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14. ABSTRACT

Prostate cancer, specifically androgen-independent prostate cancer, is the second leading cause of cancer-related deaths among men in the United States. Current treatments based on androgen ablation appear to positively select for such tumors, and the resultant highly aggressive, metastatic cancers are effectively incurable due to the absence of treatments targeting alternative pathways. Thus, as originally proposed, we have initiated a novel genome-wide shRNA screen, used successfully in previous loss-of-function screens, to uncover new therapeutic targets distinct from those essential to androgen-dependent prostate tumor proliferation and survival. To this end, we have set out to identify and characterize genes that are selectively required for the proliferation and/or cell survival of prostate cancer cells but not normal prostate epithelial cells. We expect for our research study, once fully completed, to facilitate the future discovery and/or development of small molecule inhibitors to specifically treat prostate cancer

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Introduction

Prostate cancer, specifically androgen-independent prostate cancer, is the second leading cause of cancer-related deaths among men in the United States. Current treatments based on androgen ablation appear to positively select for such tumors, and the resultant highly aggressive, metastatic cancers are effectively incurable due to the absence of treatments targeting alternative pathways. Thus, as originally proposed, we have initiated a novel genome-wide shRNA screen, used successfully in previous loss-of-function screens, to uncover new therapeutic targets distinct from those essential to androgen-dependent prostate tumor proliferation and survival. To this end, we have set out to identify and characterize genes that are selectively required for the proliferation and/or cell survival of prostate cancer cells but not normal prostate epithelial cells. We expect for our research study, once fully completed, to facilitate the future discovery and/or development of small molecule inhibitors to specifically treat prostate cancer.

Body

Background and Significance

Prostate adenocarcinoma is the most frequently diagnosed cancer and second leading cause of cancer-related deaths among men in the United States, accounting for an estimated 25% of all new cases and 10% of all cancer-related fatalities in 2008 (*1*). The risk of prostate cancer increases significantly with age, as is the case with most cancers, and the rate of incidence is expected to increase as a result of an aging baby boom generation (*2*).

Although the 5-year survival rate of diagnosed prostate cancers is over 90% (*1*), long-term survival is bleak. This is due to the fact that unless the disease has been completely surgically removed, most tumors return as aggressive, androgen-independent (AI), metastatic cancers (*3*). In contrast to localized prostate tumors, metastatic prostate cancer has only a 32% 5-year survival rate (*4*). For sustained growth and proliferation both normal and transformed prostate cells require androgens that activate androgen receptor (AR), a transcription factor that acts as a master regulator of G1-S phase progression (*5*). Current androgen ablation therapies either severely reduce testosterone production by removing the testes, or inhibit its release by administering agonists or antagonists of factors that lie directly upstream of testosterone secretion, such as luteinizing hormone-releasing hormone (LHRH) (also known as gonadotropin-releasing hormone – GnRH). This is often done in combination with inhibitors of adrenal steroidogenesis (*2*). Alternative therapies, such as estrogen administration, pure steroidal and non-steroidal direct competitive inhibitors of dihydrotestosterone (DHT), and 5α-reductase inhibitors have proved less successful (*2*).

Unfortunately, androgen ablation has been shown in several studies to be overcome by a positive selection for AI cells (*6-8*), which arise through a variety of mechanisms, including mutations that change AR specificity, sensitivity, and/or expression at a frequency of up to 50% in metastatic prostate tumors (*3*). Moreover, overexpression of insulin-like growth factor-1 (IGF-1), keratinocyte growth factor (KGF), epidermal growth factor (EGF), transforming growth factor-β (TGF-β), human epidermal growth factor-2 (HER-2/neu), and c-myc (*9-12*) can inappropriately activate AR, while tumor suppressors such as PTEN, pRb, p53 are frequently inactivated (*13*). Bcl-2, Bcl-xL, and clusterin pro-survival genes have also been implicated in the development of AI tumors (*14-16*). Additionally, because prostate cancers are heterogeneous, androgen-independent cells already present in the primary tumor may be selected for over the course of androgen ablation treatment (*3*).

The complexity of AI tumors and the universal degree to which they arise as a result of androgen ablation treatments make it critical to identify alternative targets for treatment. Although radiation and chemotherapeutic treatments have shown promise in increasing survival and time of relapse (*1*), the only drug targets exploited to date involve pathways that control androgen production and activity. Clearly, the rate-limiting process for

development of new prostate cancer therapies is target discovery. Exploiting the genetic and epigenetic differences between cancer and normal cells is a universal approach to identify new targets for cancer therapeutics, though this fundamental method has proven challenging. Sequencing efforts have revealed vast numbers of alterations in tumors, but at this point it is difficult to determine which perturbations are causative or simply benign passengers. Gain-of-function mutations in oncogenes represent the most promising targets for future drug development because of the "oncogene addiction" phenomenon, where cancer cells become abnormally dependent on the function of the oncogenes that drive tumorigenesis (*17*). In addition, we have proposed a dependency termed non-oncogene addiction in which tumors are dependent upon non-oncogene targets (*18*). For example, there is evidence of significant AR crosstalk with growth factor receptor, MAPK, cytokine, and other signaling pathways that are implicated in prostate cancer progression, and mediators of these pathways represent non-oncogene targets for prostate cancer treatment (*19*). In addition there are likely to be other targets of which we are completely unaware that could cause systems failure in the presence of a prostate cancer network of mutations. Signaling mediators that support either of these types of oncogenic and non-oncogenic pathways are ideal targets for cancer therapeutics. However, in the case of non-oncogene addiction these genes will not be mutant in tumors and therefore will be missed by sequencing approaches alone.

A major focus of my lab has been the development and application of technologies for functional genomics in mammalian cells, with a particular focus in the area of cancer biology. With our collaborators in Greg Hannon's laboratory at Cold Spring Harbor Laboratories, we have developed bar-coded shRNA libraries in retroviral vectors and Orfeome expression libraries that allow us to perform comprehensive screens for cancer relevant genes. The shRNAs become processed through the endogenous miRNA pathway and are robustly driven by a strong Pol-II promoter (*20,21*). Both enrichment and dropout screens require methods for "deconvoluting" the screen or pinpointing the bioactive clones. Using the shRNA sequence itself as a barcode, (the half-hairpin barcode) in addition to a unique 60-mer sequence in each shRNA clone, we can achieve multiple read-outs for each single vector on the microarray (Figure 1) (*22*). To date, we have performed the desired drop-out shRNA screens to look for genes required for cancer cell proliferation and survival (*22*) in addition to positive enrichment RNAi screens for checkpoint defects (*23*), cellular transformation (*24*), and ubiquitin ligases for tumor suppressors (*25*). Thus, our screening technology is a powerful and complementary approach to large sequencing efforts and is expected to provide many potential cancer drug targets (*22,26*) in an unbiased fashion, essentially asking the cancer cell to direct us to appropriate targets.

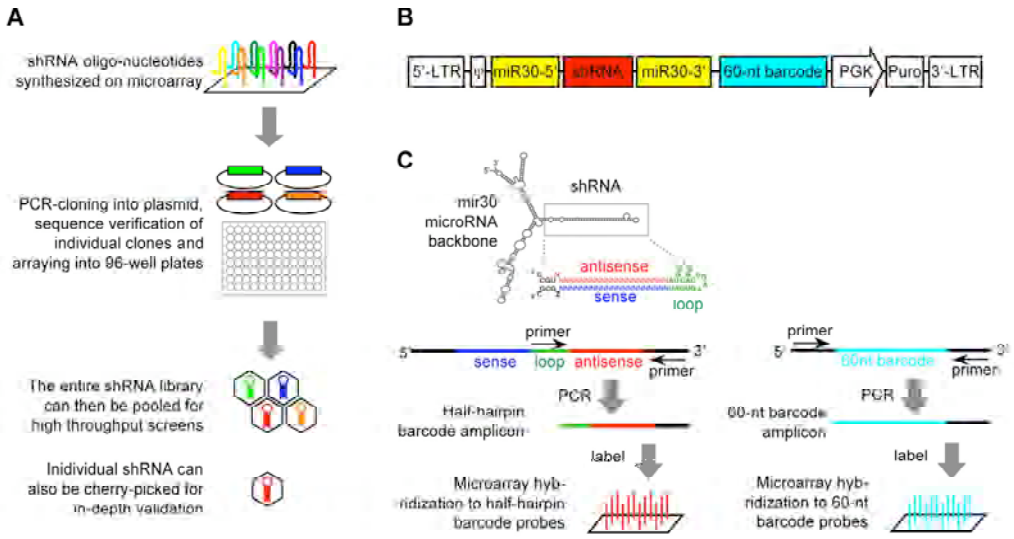


Figure 1. Overview of the Elledge-Hannon barcoded shRNA library.
A. Schematic of library construction. **B.** Schematic of the retroviral vector used to potently express the shRNA library. The shRNA is embedded in the backbone of a naturally occurring microRNA (mir30) to facilitate shRNA expression and maturation. The vector carries a puromycin-resistance marker for selection. **C.** Schematic of half-hairpin (HH) barcode and 60-nt barcode deconvolution. Because the HH and 60-nt barcodes are unique for each shRNA, the abundance of individual shRNAs in a complex pool can be tracked by hybridizing their barcode to a microarray containing the appropriate probes.

Research Strategy

We hypothesize that genetic loss-of function screens, in combination with bioinformatic and genomic analyses, can identify new targets for prostate cancer therapeutics. Here, as originally proposed, we aim to identify and characterize genes that are selectively required for proliferation and/or survival of prostate cancer cells but not normal prostate epithelial cells, which we term here as Prostate Cancer Lethal (PCL) genes.

Genome-wide shRNA screens to identify candidate PCL genes

To identify PCL genes, shRNAs can be screened either in a pool or in an individual, well-by-well format. Although pooled screening is advantageous over the latter for its highly-parallel nature and reduced cost and effort, the relative abundance of thousands of shRNAs in a pool must be measured simultaneously. Utilizing each shRNA's unique barcode for identification on microarrays, we have shown that this screening format is possible for enrichment screens (positive selection) (*24*) and lethality screens (negative selection) (*22*,*26*). Barcodes are essential for pool-based dropout screens, for example

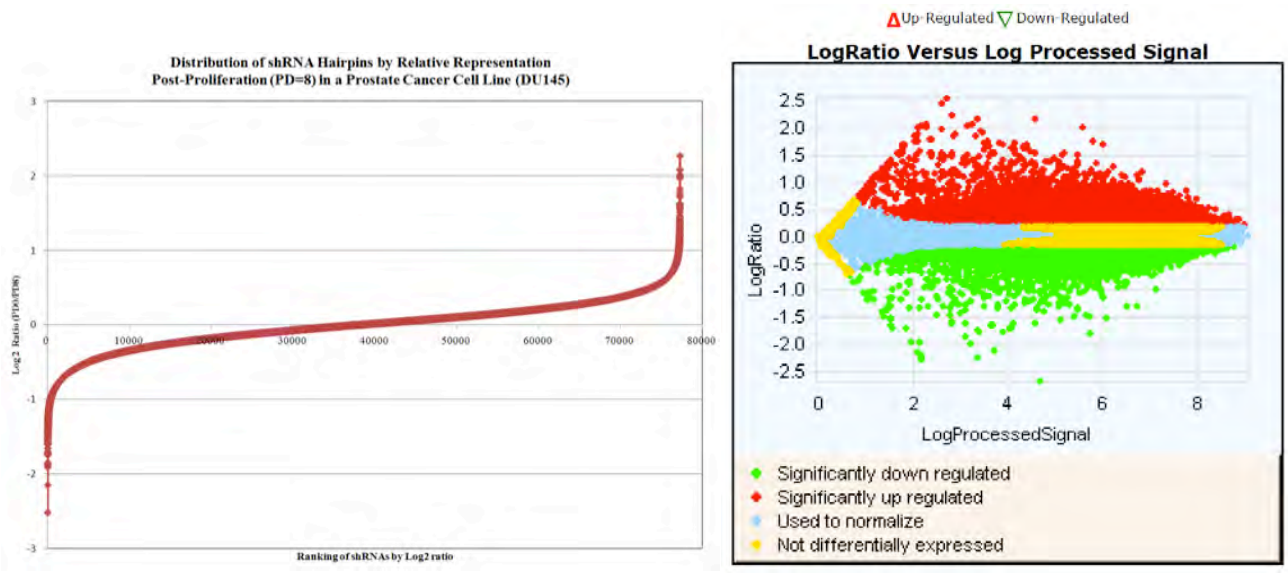
those designed to identify cell lethal or drug sensitive shRNAs (*27)*. The abundance of shRNAs targeting genes that are essential for cell viability in such lethality screens will be reduced following cell passaging and will thus "drop-out" of the shRNA population. By comparing each shRNA's abundance in an initial cell population taken shortly after retroviral shRNA library infection to its abundance in samples taken after several cell population doublings, lethal shRNAs can be identified. Moreover, comparisons between the shRNA lethality profiles of prostate cancer cells and normal human prostate epithelial cells can identify PCL genes.

We have already demonstrated the feasibility of performing multiplex shRNA screens in cancer cells in our previous functional genomics study to identify cancer proliferation genes (*22*). Consequently, we have proposed to carry out genome-wide shRNA screens with two normal human prostate epithelial cells and four prostate adenocarcinoma cell lines that represent the diversity of prostate metastases which develop. The PrEC cell line is derived from the normal prostate of a 17-year-old male (Clonetics). Because of the limited replicative lifespan of these cells, we have immortalized them with hTERT (*28*) and will refer to them as PrEC-hTERT. RWPE-1 cells are derived from the histologically normal prostate of a 54-year-old male. The cells are p53+ and pRb+ and were transfected with HPV-18 to establish an immortal cell line (*29*). The PC-3 adenocarcinoma cell line was obtained from a grade IV androgen-independent prostate cancer metastasis of the bone (*30*). It exhibits low 5α-reductase activity and is p53-, p16-, and pRb mutated (*31*). The MDA-PCa-2b adenocarcinoma cell line was also derived from a bone metastasis of androgen-independent prostate cancer. These cells are p53+ and pRb+ (*32*) and have a mutated AR (*33*). The DU-145 androgen-independent cell line was established from a prostatic metastasis of the brain (*34*) and is p53-, p16-, and pRb-mutated (*31*). LNCaP-FGC cells were isolated from a prostatic metastasis of lymph nodes. They are androgen-dependent and wild-type for p53, p16, and pRb (*31*) and express mutated AR (*35*).

As originally proposed, we have initiated genome-wide shRNA lethality screens using PrEC-hTERT, RWPE-1, PC-3, DU-145, LNCaP-FGC, and MDA-PCa-2b cell lines. RWPE-1 (cat# CRL11609), PC-3 (cat# CRL-1435), DU-145 (cat# HTB-81), LnCap-FGC (cat#CRL-1740), and MDA-PCa-2b (cat # CRL-2422) were all purchased from ATCC. To date, we have attempted to create a stable hTERT-expressing immortalized PrEC cell line; however, we have been unsuccessful in bypassing replicative senescence. For this reason, we have focused our attention, at least temporarily, on the HPV-18 –immortalized prostate epithelial cell line RWPE-1 for comparative analysis with the prostate cancer cell lines.

Comprehensive genome-wide shRNA lethality screening and data analysis has been completed for the prostate cancer cell line DU-145 (Figure 2) ; while screening for the PC-3 line has been completed but awaits half-hairpin barcode deconvolution. However, in regards to lethality screening for the RWPE-1 prostate epithelial cells, unfortunately, our protocol for retroviral infection, while suitable for most cell lines we have encountered, was not successful likely due to the unusually stringent media requirements

for these cells. We have since modified our infection protocol, taking these media requirements into account, and screening is currently ongoing.



A. B.

Figure 2. shRNA Screen in DU145 Prostate Cancer Cells
A. Distribution of shRNA hairpins by log2 ratios in DU145 prostate cancer screen. Population doublings (PD) = 8. **B.** Distribution of shRNA hairpins by log2 ratios and hybridization signal strength.

A standard screening protocol was performed using our genome-wide shRNA screening platform, described previously. Briefly, cells were infected in triplicate with ~78,000 shRNAs targeting all coding sequences in the human genome at an average of 3 shRNAs per gene, and a representation of 1000 cells per shRNA at an MOI of 2. Initial reference samples were collected 72 hours post-infection, and the remaining cells were puromycin-selected and propagated with a representation of ≥1000 cells per shRNA maintained at each passage. Infected cells were collected as the end samples after 8 population doublings (PDs). Cy3- and Cy5-labeled probes (from end or initial samples, respectively) were prepared and competitively hybridized to half-hairpin barcoded microarrays in order to measure the change in representation of each shRNA over time. Statistical analysis on similar lethality screens performed in our lab have determined that >90% of probes consistently yield signals >2-fold above the mean background of negative control probes across all triplicates, and the correlations among samples across triplicates and between the initial and end samples within each replica are high, signifying high reproducibility and maintenance of representation (*22*). These data were

analyzed using a custom statistical package based on the LIMMA method (*36*) for the analysis of 2-color cDNA microarrays. We applied the method of significance analysis for microarrays (SAM) (*37*) with a false discovery rate (FDR) of <20% and a mean log2 ratio >1 (>2-fold depletion) to identify those shRNAs consistently depleted across the triplicates in each cell line. shRNAs with a difference in the mean log2 ratios >1.5 between the normal and each prostate cancer cell line will be considered as PCL genes. The objective is to identify such PCL candidates whose loss of function reduces cell growth and viability in prostate cancer cell lines, but does not affect the viability of normal prostate cells. Identifying PCL genes that are either common across all prostate cancer cell lines tested or unique to a subset of prostate cancer cell lines with similar pathologies may also reflect common pathways required to support tumor growth and represent attractive drug targets.

Since we have not finished generating a shRNA lethality profile for a normal prostate epithelial cell line, we have not strictly been able to perform a comparative analysis for the identification of PCL. Still, we have been able to compare the shRNA lethality profile of the DU-145 prostate cancer line with the lethality profile of a normal human mammary epithelial cell line previously generated in the laboratory. As shown for the top fifty drop-outs for DU145 prostate cancer cells (Figure 3) , 41 out of the 50 shRNA hits differ from human mammary epithelial cells (HMECs) in mean log2 ratio by less than 1.5 log2 units. This suggests that our screen in DU145 cells is successfully uncovering genes that are fundamentally required for proliferation and survival regardless of cell type. What is potentially more interesting are the 9 out of 50 top shRNA drop-outs for DU145 that differ for that of HMECs by more than 1.5 log2 units. Indeed, these hits may represent prostate-specific requirements for proliferation and survival, or in addition, they may represent sought-after PCL genes.

HMEC	DU145	*Difference*	ID-short
-1.25	-2.51	1.3	LOC642179
-2.47	-2.15	0.3	RPL39
-1.14	-1.91	0.8	LOC390729
-1.12	-1.87	0.8	FLJ13224
-1	-1.85	0.8	NA
0.5	-1.74	2.2	LOC285326
-1.95	-1.74	0.2	GLRX
-1.12	-1.72	0.6	CNOT1
-0.06	-1.70	1.6	CAMTA1
-1.01	-1.65	0.6	NA
-1.52	-1.60	0.1	CDC25B
0.22	-1.60	1.8	NF2
-2.1	-1.59	0.5	NA
-0.37	-1.59	1.2	PSMD3
-1.07	-1.57	0.5	ZNF45
-2.39	-1.55	0.8	B2M
0.5	-1.55	2.0	NA
-0.08	-1.53	1.4	NR6A1
1.08	-1.52	2.6	CIDEB
0.54	-1.51	2.1	NID1
-2.72	-1.51	1.2	POLR2E
-0.77	-1.50	0.7	NA
-2.11	-1.49	0.6	EYA4
0.65	-1.47	2.1	ABAT
-1.44	-1.46	0.0	NA
-1.6	-1.45	0.2	CRSP2
-0.98	-1.43	0.5	GCA
-0.35	-1.43	1.1	SPATA21
-2.1	-1.43	0.7	BRSK2
-1.67	-1.42	0.2	RASSF7
-0.52	-1.42	0.9	SHH
0.04	-1.42	1.5	NA
-0.73	-1.41	0.7	NA
-1.77	-1.41	0.4	MYOC
-2.48	-1.40	1.1	S100A9
-1.99	-1.39	0.6	UBR4
-0.05	-1.38	1.3	TMEM50B
-1.7	-1.38	0.3	NA
-0.27	-1.38	1.1	TNIK
-0.24	-1.38	1.1	P2RX7
-0.68	-1.37	0.7	MPZ
-2.15	-1.37	0.8	NA
-0.33	-1.37	1.0	SEPHS2
-0.8	-1.35	0.6	ICAM2
0.08	-1.34	1.4	STAM
-0.49	-1.34	0.9	SIRT4
-0.17	-1.34	1.2	MCM6
-0.11	-1.34	1.2	ADSS

Figure 3. Comparative Analysis: DU145s vs. HMECs
Mean log2 ratios for shRNA hairpins were ranked from lowest (drop-outs) to highest (enrichments) for the DU145 screen. The log2 ratios for these top fifty shRNA drop-outs in DU145s were compared against the log2 ratios for the corresponding shRNAs in a HMEC screen. Orange rows represent mean log2 differences >1.5. Gray, <1.4

Investigation of the contribution of PCL candidates to signaling networks that mediate proliferative and pro-survival cues to support prostate tumorigenesis.

Validation of candidate genes and the exclusion of shRNAs exhibiting off-target effects.

As we originally proposed, we will validate PCL candidates with secondary assays using additional independent shRNAs against the target gene. To validate each gene, PrEC-hTERT (once engineered), RWPE-1, PC-3, DU-145, LNCaP, and MDA-PCa-2b cells will be plated in 96-well plates and individually transduced with single shRNAs. Cell viability will be assessed several days later with the CellTiter-Glo Assay. We will also assess the effect of individual shRNAs on normal prostate and prostate cancer cells using the multi-color competition assay (MCA) (*38*). siRNAs will additionally be used for further validation. Candidates that validate in the secondary assays as selectively lethal in prostate cancer will have shRNA and siRNA knockdown efficiency in the normal and cancer cell lines determined using RT-PCR and Western blotting. Rescue experiments with shRNA-resistant cDNAs can also be performed to ensure that cDNA expression of the target gene reverses the effect of a shRNA on cell viability. Only genes with multiple shRNAs or siRNAs whose effect on cell viability correlates with mRNA or protein knockdown, and show similar knockdown efficiencies between the two or more cell types will be considered.

Bioinformatic analysis to identify candidates for future mechanistic studies.

One significant challenge associated with large scale screens is prioritization of candidate genes. We expect to identify a group of PCL genes, which are required for viability for some of the lines while others will be required for viability of all prostate cancer lines but not normal prostate epithelial cells tested. To prioritize candidate genes for further study, we will concentrate first on candidate genes that score with multiple shRNAs in the original screen. Moreover, available databases of genomic, transcriptional, functional, and protein-protein interaction information will be utilized to identify potential oncogenes by searching for genes amplified (*39,40*), mutant (*41,42*), or overexpressed in these cell lines and human breast cancers (*39,43*). Because we expect that some genes will fall into previously identified signaling pathways, we will also employ gene ontology (GO) categories (*44,45*), protein-protein interactions and Ingenuity analysis (*46*) to identify signaling networks that might connect genes that are not prioritized by the above criteria.

Determination of the mechanisms of action of validated candidate genes whose loss of function selectively impair prostate cancer cell viability.

shRNAs that either reduce cell proliferation rate or increase the death rate should cause, theoretically, a decrease in cell number. We will use propidium iodide (PI) staining and FACS analysis to determine cell cycle profiles, investigate cell cycle arrest, and investigate whether an increase in the sub-G1 (apoptotic) population is observed upon shRNA expression. TUNEL staining and BrdU incorporation assays will be also

performed to determine if candidate shRNAs reduce viability by increasing apoptosis or decreasing proliferation, respectively. Based on literature searches, protein domain information, and potential sites for protein modification, candidate genes will be further investigated to identify related signaling networks and the mechanism by which each candidate supports cancer cell proliferation and/or survival. Those that are required by a subset of prostate cancer cells, particularly the androgen independent cells, will be investigated for the potential perturbation of these oncogenic pathways.

Correlation of candidate gene expression, amplification or deletion with tumor grade and patient prognosis.

Accurately determining the *in vivo* prognostic significance of candidate genes is key to discovering potential drug targets, as well as in identifying possible biomarkers to predict disease progression. Tissue microarray slides (TriStar Technology Group, Rockville, MD) containing 2800 prostate cancer specimens with documented clinical histories will be separately immunostained with antibodies against individual candidate genes and counterstained with haematoxylin. Slides will be scanned and scored using the following scale: 0 – negative staining; 1 – weak staining, <50% of individual cell or <5% of all cells; 2 – moderate staining, >50% of individual cell or >5% of all cells (*47*). Tissue samples will be categorized based on histopathological factors, and the correlation significance will be determined using Pearson's chi-squared test. Kaplan-Meier plots will be constructed to establish an association between patient survival and candidate gene expression levels. The COX proportional hazards test for multivariate analysis will also be applied to exclude effects of other prognostic factors (tumor state, histological grade, etc.) on candidate gene expression. Candidates with a high correlation of gene expression levels and tumor grade and patient survival will represent strong candidates for further drug target and tumor diagnosis testing. Such analysis has already revealed strong prognostic significance of gene expression levels in prostate tumors (*47*) and numerous other cancers such as breast (*48*), lung (*49*) and melanoma (*50*).

These screens that we have we proposed and initiated have the potential to identify genes that support the survival and proliferation of cancer cells. We expect to identify oncogenes to which the cancer cells is addicted and cannot live without. In addition we expect to identify support pathways that allow oncogenes to function but which are themselves not oncogenes. Finally we expect to find totally unanticipated genes and pathways that are inconsistent with the oncogenic state, whether it be androgen dependent signaling, aneuploidy, cell cycle regulation, oxidative stress or other stress support pathways essential for the unstable genome to survive. These genes will represent unbiased targets for cancer cell directed therapies in the future.

Summary

Statement of Work

Year 1.

Task 1 (Months 1-3)
In the first 3 months, we will generate the PrEC + hTERT cell line using a blastocydin-selectable retroviral construct. We will characterize this cell line to ensure stable expression and activity of hTERT using the telomeric repeat amplification protocol (TRAP) assay. Telomere length of PrEC-hTERT cells compared to late passage PrEC cells will be determined using the terminal restriction fragment length (TRL) assay.

To date, we have attempted to create a stable hTERT-expressing immortalized PrEC cell line; however, we have been unsuccessful in bypassing replicative senescence. For this reason, we have focused our attention, at least temporarily, on the HPV-18 – immortalized prostate epithelial cell line RWPE-1 for comparative analysis with the prostate cancer cell lines.

Task 2 (Months 3-15)
We will perform genome-wide shRNA lethality screens on PrEC-hTERT, RWPE-1, PC-3, DU-145, LNCaP-FGC, and MDA-PCa-2b cells to generate lethality profiles for each cell line.

Comprehensive genome-wide shRNA lethality screening and data analysis has been completed for the prostate cancer cell line DU-14: while screening for the PC-3 line has been completed but awaits half-hairpin barcode deconvolution. In regards to lethality screening for the RWPE-1 prostate epithelial cells, unfortunately, our protocol for retroviral infection, while suitable for most cell lines we have encountered, was not successful likely due to the unusually stringent media requirements for these cells. We have since modified our infection protocol, taking these media requirements into account, and screening is currently ongoing. We fully expect to complete screening and analysis for all of these cells by month 15.

Year 2.

Task 3 (Months 13-24)
We will perform validation experiments with multiple individual shRNAs and siRNAs against candidate PCLs in CellTiter-Glo and MCA assays in order to confirm candidates that reduce cell growth and viability in prostate cancer cell lines, but do not affect normal prostate cell viability. We will also determine which candidate PCLs identified in the primary screen are false positives due to off-target effects.

Task 4 (Months 13-24)
For candidates that validate in the secondary assays, we will use RT-PCR and Western blottingto examine shRNA and siRNA knockdown efficiency. Only genes with a correlation between mRNA or protein knockdown and multiple shRNAs or siRNAs affecting cell viability in two or more prostate cancer cell types will be considered for further study.

Task 5 (Months 18-24)
We will generate a list of validated PCL genes for further analysis and study from the data generated in tasks 3 and 4.

Year 3.

Task 6 (Months 24-30)
We will perform bioinformatics analysis to identify potential oncogenes by searching for genes amplified, mutated, or overexpressed in these cell lines and human breast cancers. This will allow us to prioritize validated PCL candidates for further mechanistic studies. Gene ontology (GO) categories, protein-protein interactions and Ingenuity analysis will also be utilized to identify signaling networks that might connect genes within the list.

Task 7 (Months 24-30)
We will determine whether validated PCL genes negatively affect cell viability (increase apoptosis) or cell proliferation (decrease replication) by using propidium iodide staining, FACS analysis, TUNEL staining and BrdU incorporation assays.

Task 8 (Months 30-36)
We will determine expression levels of validated PCL genes in a spectrum of prostate cancer tissue samples using a tissue microarray. This will determine if there is a prognostic correlation between gene expression and tumor histopathology and patient survival, as well as help identify strong drug target candidates.

Key Research Accomplishments

1. Completion of a shRNA lethality profile for androgen-independent prostate cancer cell line DU145.
2. Comparative analysis of lethality profiles for DU145 prostate cancer cells versus a normal human mammary epithelial cell line.

Reportable Outcomes

Not applicable at this time.

Conclusions

Generation of an hTert-immortalized "normal" prostate epithelial cell line
We have attempted to engineer a stable hTERT-expressing normal PrEC cells; however, we have been unable to bypass replicative senescence in these cells to generate the required immortalized cell line for our studies. We are currently troubleshooting the problem. In the meantime, we have focused our attention, on the HPV-18 –immortalized prostate epithelial cell line RWPE-1 for comparative analysis with the prostate cancer cell lines.

Progress on genome-wide shRNA lethality screens for normal prostate epithelial cells and prostate carcinoma cell lines.
Comprehensive genome-wide shRNA lethality screening and data analysis has been completed for the prostate cancer cell line DU-14: while screening for the PC-3 line has been completed but awaits half-hairpin barcode deconvolution. In regards to lethality screening for the RWPE-1 prostate epithelial cells, unfortunately, our protocol for retroviral infection, while suitable for most cell lines we have encountered, was not successful likely due to the unusually stringent media requirements for these cells. We have since modified our infection protocol, taking these media requirements into account, and screening is currently ongoing.

Since we have not finished generating a shRNA lethality profile for a normal prostate epithelial cell line, we have not strictly been able to perform a comparative analysis for the identification of PCL. Still, we have been able to compare the shRNA lethality profile of the DU-145 prostate cancer line with the lethality profile of a normal human mammary epithelial cell line previously generated in the laboratory. From the comparative analysis of these two screens, we have potentially uncovered genes that are fundamentally required for proliferation and survival regardless of cell type. Perhaps more interestingly, we have identified DU145-specific shRNA drop-outs. Indeed, these candidate genes may represent prostate-specific requirements for proliferation and survival, or in addition, they may represent sought-after PCL genes. The upcoming year will be used to validate and characterize these PCL gene candidates.

References:

1. A. Jemal *et al., CA Cancer J Clin* **58**, 71 (2008).
2. S.R. Denmeade, J. T. Isaacs, *Nat Rev Cancer* **2**, 389 (2002).
3. B.J. Feldman, D. Feldman, *Nat Rev Cancer* **1**, 34 (2001).
4. L.A.G. Ries *et al., SEER Cancer Statistics Review* National Cancer Institute, Bethesda, MD (1979-2005).
5. S.P. Balk, K. E. Knudsen, *Nucl Recept Signal*, **6**, 1 (2008).
6. M. Taplin *et al., Cancer Res*, **59**, 2511 (1999).
7. Z. Culig *et al., Prostate*, **35,** 63 (1998).
8. N. Craft *et al., Cancer Res*, **59**, 5053 (1999).
9. D. Bernard *et al., J Clin Invest*, **112**, 1724 (2003).
10. C.D. Chen *et al., Nat Med*, **10**, 33 (2004).
11. P. Rocchi *et al., Oncogene*, **23**, 9111 (2004).
12. S. Kiyama *et al., Cancer Res*, **63**, 3575 (2003).
13. D.J. Mulholland *et al., Oncogene*, **25**, 329 (2006).
14. M. Gleave *et al., Clin Cancer Res*, **5**, 2891 (1999).
15. H. Miyake *et al., Int J Cancer*, **86**, 855 (2000).
16. H. Miyake *et al., Cancer Res*, **60**, 170 (2000).
17. I. B. Weinstein and A. Joe, *Nat Clin Pract Oncol*, **3**, 448 (2006).
18. N. L. Solimini *et al., Cell* **130**, 986 (2007).
19. M. Kaarbo *et al., Bioessays*, **29**, 1227 (2007).
20. R. A. Dickins *et al., Nat. Genet.* **39**, 914 (2007).
21. F. Stegmeier *et al., Proc. Natl. Acad. Sci. U S A* **102**, 13212 (2005).
22. M. R. Schlabach *et al., Science* **319**, 620 (2008).
23. D. M. Draviam *et al., Nat Cell Biol*, **9**, 556 (2007).
24. T. F. Westbrook *et al., Cell* **121**, 837 (2005).
25. F. Stegmeier *et al., Nature* **446**, 876 (2007).
26. J. M. Silva *et al., Science* **319**, 617 (2008).
27. V. N. Ngo *et al., Nature* **441**, 106 (2006).
28. G. Shao, *et al., Mol Carcinog*, [Epub ahead of print] (2008).
29. D. Bello *et al., Carcinogenesis*, **18**, 1215 (1997).
30. M. E. Kaighn *et al., Invest Urol*,**17**, 16 (1979).
31. C. Park *et al., Int J Mol Med*, **17**, 715 (2006).
32. N. M. Navone *et al., Clin Cancer Res*, **6**,1190-7 (2000).
33. X. Y. Zhao *et al., J Urol*, **162,** 2192 (1999).
34. K. R. Stone *et al., In. J Cancer* **21**, 274 (1978).
35. J. Veldscholte *et al., Biochem Biophys Res Commun*, **173**, 534 (1990).
36. G. K. Smyth, T. Speed, *Methods* **31**, 265 (2003).
37. V. G. Tusher, R. Tibshirani, G. Chu, *Proc. Natl. Acad. Sci. U S A* **98**, 5116 (2001).
38. A. Smogorzewska *et al., Cell* **129**, 289 (2007).
39. R. M. Neve *et al., Cancer Cell* **10**, 515 (2006).
40. Copy Number Analysis in Cancer, http://www.sanger.ac.uk/cgibin/genetics/CGP/CGH_home.cgi.
41. T. Sjoblom *et al., Science* **314**, 268 (2006).

42. Catalogue of Somatic Mutations in Cancer (COSMIC), http://www.sanger.ac.uk/genetics/CGP/cosmic/.
43. K. Chin *et al.*, *Cancer Cell* **10**, 529 (2006).
44. AmiGO, http://amigo.geneontology.org.
45. The Gene Ontology Consortiumm, *Nucleic Acids Res* **36**, D440 (2008).
46. Ingenuity Pathway Analysis, www.ingenuity.com.
47. B. Lin *et al.*, *Clin Cancer Res* **14**,1397 (2008).
48. H. J. Han *et al.*, *Nature* **452**, 187 (2008).
49. H. Zheng *et al. Anticancer Res* **27**, 3561 (2007).
50. J. Rangel *et al.*, *J Clin Oncol* **24**, 4565 (2006).

STEPHEN J. ELLEDGE

Current & Pending Support:

HHMI (Stephen J. Elledge) 09/01/06-08/31/10 1.2 cal. mo.
Howard Hughes Medical Institute $837,790/yr

Cell Cycle Control During Development

The major goal of this project is to analyze how cell cycle control is regulated during development. We have used this support to our efforts to generate RNAi libraries for exploring functions of human genes.
Overlap: None

U19 AI067751-01 (Alan D. D'Andrea) 08/31/05 – 07/31/2010 1.2 cal. mo.
NIH/NIAID $357,079/yr

Center for Medical Countermeasures Against Radiation

This project aims to look for genes that confer radiation sensitivity on hematopoetic stem cells with the goal of finding genes whose inhibition would provide resistance to radiation damage and oxidative stress.
Overlap: None

R37 GM044664 (Stephen J. Elledge) 07/01/08 – 06/30/13 2.4 cal. mo.
NIH-NIGMS $350,000/yr

Genetics of Cell Cycle and DNA Damage Regulation in Yeast

The major goal of this project is to understand the cellular response to DNA damage and replication blocks in *S. cerevisiae*.
Overlap: None

W81XWH-08-1-0638 (Stephen J. Elledge) 09/01/08 – 08/31/13 1.2 cal. mo.
Department of Defense – Army Innovator $3,500,000 over 5 years

Using Genetics and Genomics for the Detection and Treatment of Breast Cancer

This project has 3 major aims. Genetic screens to identify genes regulating the proliferation and survival of breast cancer cells, genes whose inhibition will enhance chemotherapy for breast cancer, and development of a sensitive method for detecting breast cancer specific proteins in blood and urine.
Overlap: None

Grant Number 51244 (Stephen J. Elledge) 11/01/08 – 10/31/11 1.2 cal. mo.
Bill & Melinda Gates Foundation $5,700,000 over 3 years

Identification of Host Factors Required for HIV Infection using Functional Genomics

The purpose of the grant is to use the revolutionary advances in mammalian genetics to identify host factors that HIV depends upon for its survival, and in so doing provide systems level understanding of viral-host interactions that will stimulate new ideas and endeavors.
Overlap: None

W81XWH-08-1-0638 (Stephen J. Elledge) 09/01/09 – 10/31/12 1.2 cal. mo.
Department of Defense – Army Idea Award $656,224 over 3 years

Identification of Genes Required for the Survival of BRCA1-/- Cells

This project aims to perform genome-wide shRNA screens to identify genes that are selectively required for proliferation and survival of breast cancer cells with *BRCA1* loss-of-function mutations but not breast cancer cells expressing wild-type, functional *BRCA1*. Identification of such genes will provide new therapeutic targets for breast cancers harboring *BRCA1* mutations.
Overlap: None

1R33CA140039-01 (Stephen J. Elledge) 08/01/09 – 07/31/12 1.2 cal. mo.
NIH/NCI $365,091

A Multiplex Genome-Wide shRNA Screening Platform for Cancer-Lethal Discovery
The purpose of this grant is to develop an ultra-high throughput RNA interference (RNAi) screening platform for the identification of cancer-lethal genes. This screening platform will enable the rapid, genome-wide screening of large numbers of cancer and normal lines to identify genes that are selectively required for cancer cell viability and therefore might serve as potential cancer drug targets.
Overlap: None

W81XWH-09-1-0211 (Stephen J. Elledge) 05/15/09 – 06/14/12 1.2 cal. mo.
Department of Defense – Army Idea Award $656,090 over 3 years
Identification of Genes Required for the Survival of Prostate Cancer Cells
This project aims to identify genes essential for the viability of prostate cancer cells but not for non-transformed, normal prostate epithelial cells. The identified genes will subsequently be investigated for their contribution to signaling networks that mediate proliferation and survival in support of prostate tumorigenesis.
Overlap: None

Pending:

W81XWH-09-MSRP-DMVA (Stephen J Elledge) 09/30/10 - 09/29/2011 .60 cal .mo.
Department of Defense-Multiple Sclerosis Research Program $90,326
PhIP-Seq for Autoantibody Profiling in MS
The primary aim is to identify autoantibody biomarkers specific for MS, it is possible that our findings may have therapeutic implications as well. There has been increasing interest in inducing tolerance to MS-specific antigens.
Overlap None